

Supporting information

Calcium Doped Mesoporous Silica Nanoparticles as Efficient Alendronate Delivery Vehicles

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Experimental details

Drug loading

Several batches of the synthesized Ca-MSNs (200 mg) were immersed in 10 mL of a 20 mg/mL AL solution at pH 4.8 ($\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer, 50 mM) at room temperature under vigorous stirring. The process was continued overnight. The resulting suspension was centrifuged at 8000 rpm for 20 min, washed with distilled water for several times. After that, the suspension was centrifuged and dried at room temperature under vacuum over night. The AL-loaded samples were denoted as Ca-MSNs-AL. The effective AL storage capacities of the Ca-MSNs were quantified by measuring the total amount of elemental phosphorus in the supernatant before and after the loading by means of ICP-AES. As a control experiments, undoped MSNs were also loaded with AL under the same conditions with Ca-MSNs loading procedures.

Drug Release

The in vitro AL delivery tests were performed by soaking the 50 mg Ca-MSNs-AL in 15 mL of NaCl 0.9% solution at the pH of 7.4 (the pH was buffered using a 50 mM aqueous solution of tris(hydroxymethyl)aminomethane). These suspensions were placed into dialysis bags with a molecular weight cut-off of 3500 Da, and subsequently placed in a beaker containing 50 ml of 0.9% NaCl aqueous solution with the same pH conditions. The volume of the dissolution media was maintained at 50 mL while constant stirring (~100 rpm) at 37 °C. At a predetermined time, 0.5 mL of suspension was removed. Spectrophotometric determination of alendronate sodium was achieved through their oxidation using excess ceric (IV) sulphate in the presence of 0.5M sulphuric acid at room temperature. The amount of the consumed ceric equivalent to the concentration of AL was determined by measuring the absorbance of ceric solution (as blank) against the test solution at 320 nm.¹ These measurements gave the information on the quantity of released AL.

Characterization and measurements

X-ray diffraction (XRD) patterns were collected with Bruker D8 using Cu K α radiation (40 kV, 40 mA). N₂ adsorption-desorption isotherms were obtained on NOVA 4200e at 77 K under a continuous adsorption condition. All samples were pretreated for 12 h at 393K under nitrogen before measurements. The Brunauer-Emmett-Teller (BET) method was utilized to calculate the specific surface areas using adsorption data in a relative pressure range from 0.2 to 0.4. By using the Barrett-Joyner-Halenda (BJH) model, the pore volumes and pore size distributions were derived from the adsorption branches of isotherms, and the total pore volumes were estimated from the adsorbed amount at a relative pressure P/P₀ of 0.99. UV-visible absorption spectra were recorded on a Shimadzu

UV-vis 3101 spectroscopy. Transmission electron microscopy (TEM) and energy dispersive spectroscopy (EDS) was conducted on a JEM 2100F electron microscope operated at 200 kV. Scanning electron microscopy (SEM) was performed on JEOL JSM6700F electron microscope. The calcium contents in the prepared samples were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Solid state NMR spectra were recorded on a Bruker AV400 spectrometer at a resonance frequency of 100.53 MHz with a contact time of 1.5 ms.

In vitro biocompatibility of Ca-MSNs

For the biocompatibility studies of Ca-MSNs with HeLa cells, cells were seeded in a 96-well plate at a density of 10^4 cells per well and cultured in 5% CO₂ at 37 °C for 24 h. Then, the above prepared Ca-MSNs were added to the media, and the cells were incubated in 5% CO₂ at 37 °C for 24 h and 48 h. The concentrations of Ca-MSNs were set as 20, 140, 260, 380 and 500 µg/mL, respectively. Cell viability was determined by the standard 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.

In vitro cytotoxicity of Ca-MSNs-AL

For the cytotoxicity analysis of free AL, Ca-MSNs-AL against HeLa cells, cells were seeded in a 96-well plate at a density of 10^4 cells per well and cultured in 5% CO₂ at 37 °C for 24 h. Then, free AL dispersed in DMSO and Ca-MSNs-AL were added to the media, and the cells were incubated in 5% CO₂ at 37 °C for 24 h and 48 h. The equivalent concentrations of AL were 5, 25, 50, 75 and 150 µM, respectively. Cell viability was determined by MTT assay. As a control experiment, the cytotoxicity of MSNs-AL was also measurement under the same conditions as Ca-MSNs-AL. The statistical analysis of experimental data utilized the Student's t-test. A p-value of less than 0.05 was considered statistically significant. Each data point is represented as mean ± standard deviation (SD) of eight independent experiments (n = 8, n indicates the number of wells in a plate for each experimental condition).

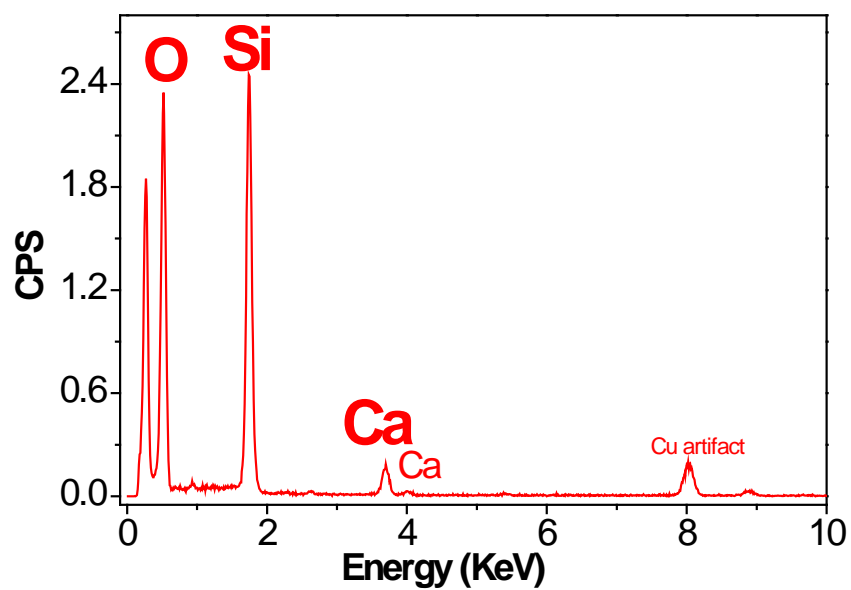


Fig. S1 EDS spectrum of the synthesized Ca-MSNs.

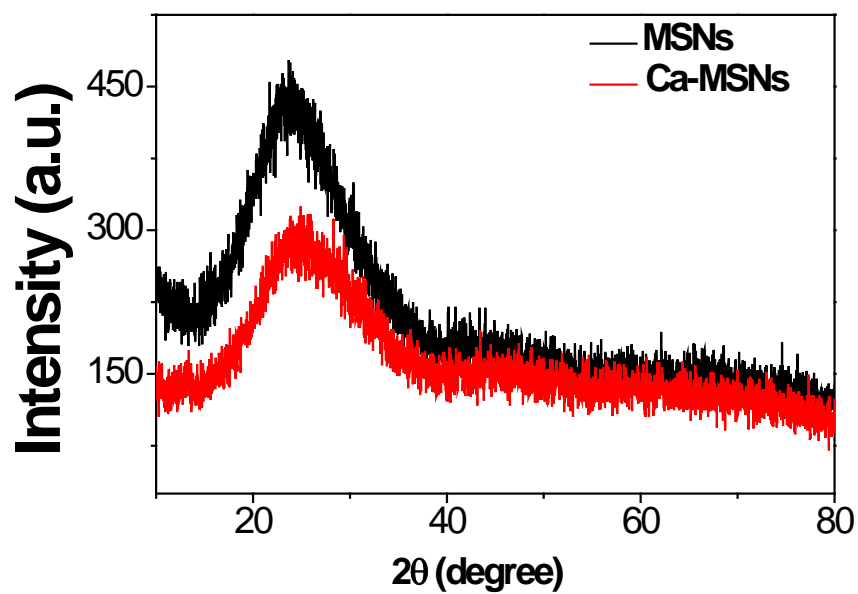


Fig. S2 Wide angle XRD patterns of the synthesized MSNs and Ca-MSNs.

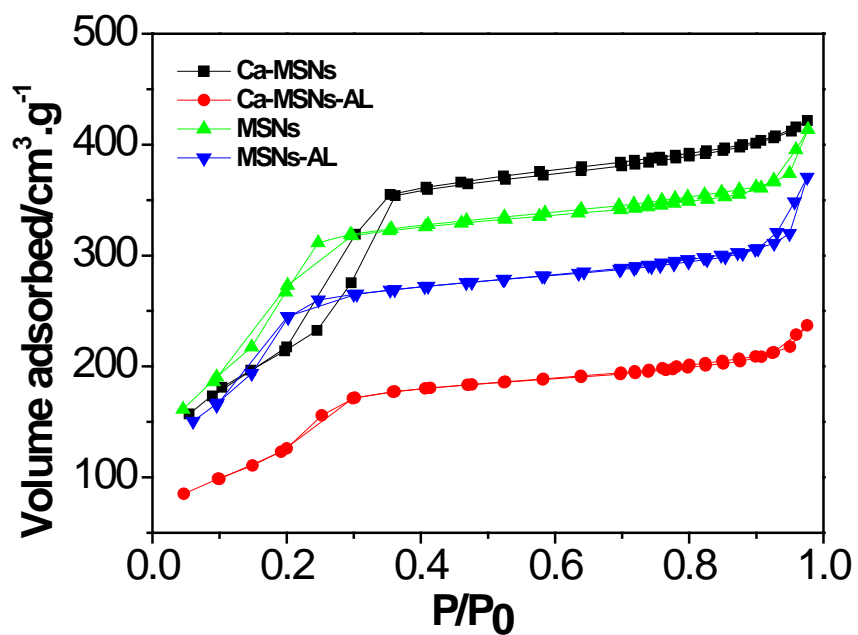


Fig. S3 Nitrogen adsorption/desorption isotherms of Ca-MSN, Ca-MSN-AL, MSN and MSN-AL, respectively.

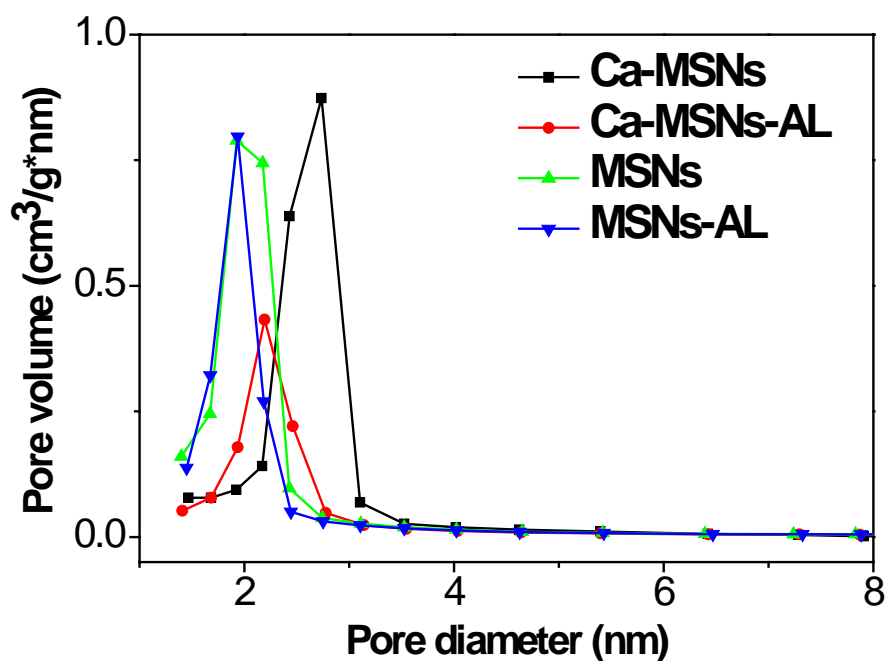


Fig. S4 Pore size distribution of the synthesized samples.

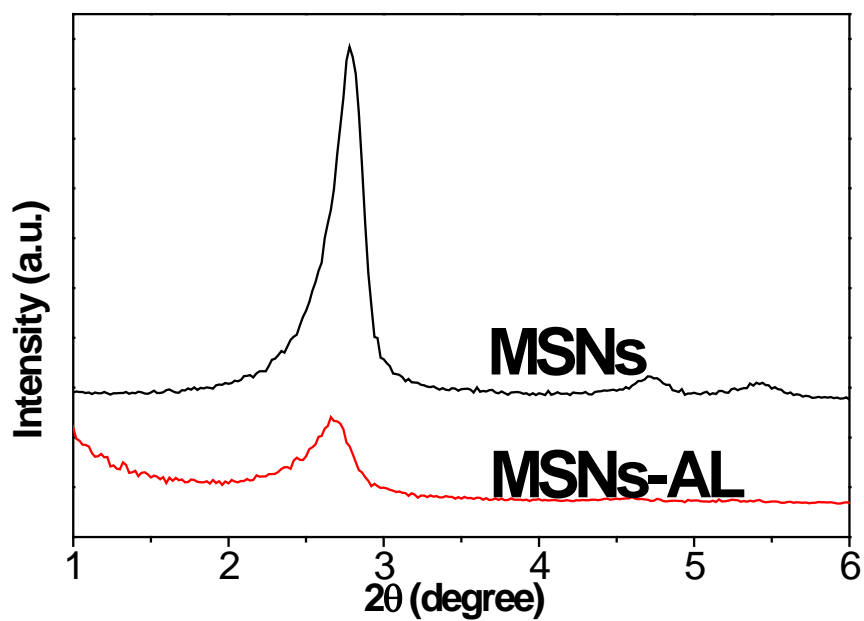


Fig. S5 Small angle XRD patterns for the pure MSNs before and after drug loading.

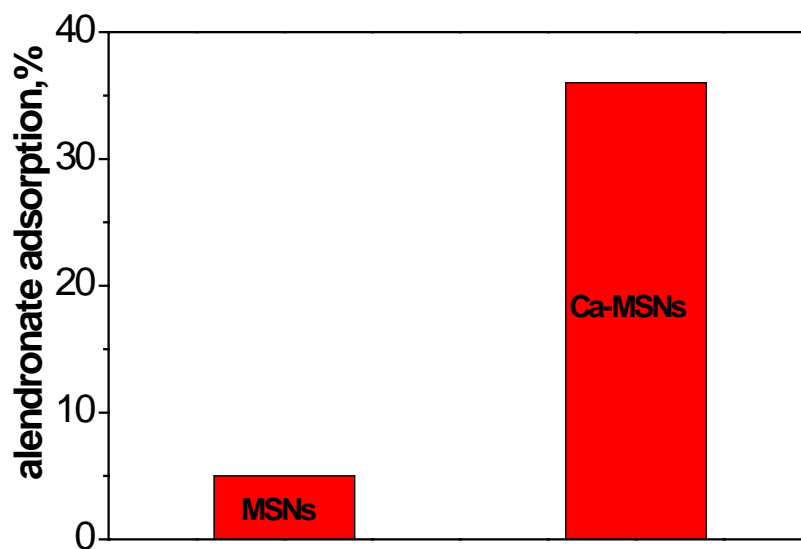


Fig. S6 Comparative profiles of the maximum loading of AL in Ca-MSNs and MSNs.

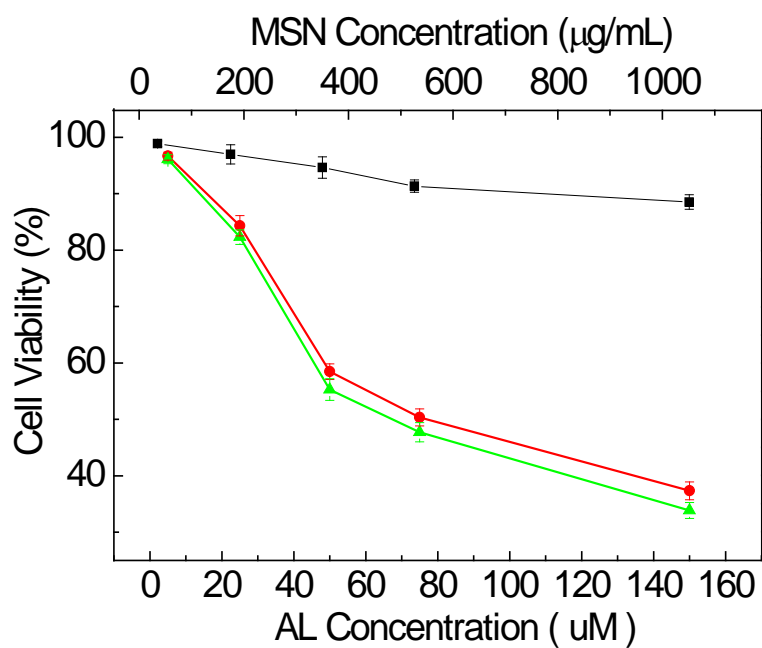


Fig. S7 Comparative cytotoxicity profiles of free AL (Red), MSNs-AL (Green) and parent MSNs (Black) against HeLa cell lines after culture for 1 day. The top abscissa is for the parent MSNs while the bottom one is for free AL and MSNs-AL.

Reference:

- 1) E. A. Taha and N. F. Youssef, *Chem. Pharm. Bull.*, 2003, **51**, 1444.